



The effect of nitrate addition on abundance of *nirK*, *nirS* and *gln* genes in acidified Norway spruce forest soil

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The denitrification is the main biotic process leading to losses of fixed nitrogen as well as removal of excess of nitrate (NO_3^-) from the soil environment. The reduction of NO_2^- to nitric oxide (NO) distinguishes the “true” denitrifiers from other nitrate-respiring bacteria. This reaction is catalyzed by two different types of nitrite reductases, either a cytochrome cd1 encoded by *nirS* gene (*nirS* denitrifiers) or a Cu-containing enzyme encoded by *nirK* gene (*nirK* denitrifiers). The *nirS* denitrifiers are located mostly in rhizosphere, while the *nirK* denitrifiers are more abundant in bulk soil. These two groups can be also classified as markers of denitrification.

Glutamine synthetase is one of the main bacterial NH_4^+ assimilating enzymes; it is coded by *glnI* gene. Glutamine synthetase is mostly active when N is the limiting factor for bacterial growth. There is recent evidence that the activity may be affected by the presence of alternative N source (i.e. NO_3^-). However, in anaerobic condition NO_3^- can be used also by the denitrifying bacteria so there may be strong competition for this nutrient.

The laboratory experiment was performed to evaluate the effect of nitrates (NO_3^-) on abundance of *nirK*, *nirS* and *gln* gene copy numbers. The amount of NO_3^- corresponded to the actual atmospheric depositions on experimental sites in the Bohemian Forest. Litter organic layer (0-5cm of soil) was used for laboratory incubation experiment. Four replicates of control (no addition of NO_3^-), and NO_3^- addition were incubated anaerobically for one month. After the incubation DNA was extracted and the number of *nirK*, *nirS* and *gln* gene copies was determined using qPCR (SYBRGreen methodology). Results showed that the addition of NO_3^- significantly increased the number of *nirK* and *nirS* denitrifiers from 5.9×10^6 to 1.1×10^7 and from not detectable amount to 1.4×10^6 , respectively. The *gln* gene copy number was also higher after NO_3^- addition. However, the difference was not statistically significant.